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Bacteriology Department.

Studies on Hog Cholera and Preventive Treatment.

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Bulletin No. 157—November, 1908.

Bacteriology Department.

WALTER E. KING, M. S., Bacteriologist.

Studies on Hog Cholera and Preventive Treatment.

ACKNOWLEDGMENT.

Dr. Chas. T. McClintock, director of the biological laboratory of Parke, Davis & Co., Detroit, Mich., has conducted a continuous line of investigation on hog cholera for the last twelve years. After a series of studies on the *Bacillus cholera suis*, including a study of the value of agglutination for the diagnosis of the disease, he began an aggressive line of work with the filterable virus. He made many attempts to attenuate virulent hog-cholera blood, using the various physical and chemical agents, particularly those suggested by his own work and that of others on smallpox vaccine. All of these attempts were unsuccessful. At that time it occurred to Doctor McClintock that Jenner's work might apply to vaccination against hog cholera; that even though we had no other animal susceptible to the disease, a residence in the body of another animal might attenuate the hog-cholera virus without totally destroying it.

The opportunity to carry on the work outlined in the following pages is largely, if not wholly, due to Doctor McClintock. Furthermore, the method of producing the experimental vaccine, such as has been used in the following experiments, was first suggested by Doctor McClintock.

NOTE.—The plates referred to in this Bulletin are photo reproductions and may be found grouped at the end of the article.

The studies on the value of this vaccine and methods of producing the same have been carried out jointly. The earlier experiments were made in the research department of Parke, Davis & Co., Detroit, Mich., and the later ones at the Kansas State Experiment Station.

I.

INTRODUCTION.

The suppression of the disease, hog cholera, a problem which is recognized as one of great importance to the agricultural interests of the United States, has received considerable attention during the last twenty years. At the present time few problems are as intimately associated with the agricultural economy of the state of Kansas as that which is related to the practical eradication of this animal disease.

Reports from various parts of the state give conclusive evidence of the great prevalence of hog cholera at this time. Hundreds of Kansas farms have become infected and thousands of hogs have perished during the past few months from this disease. The increased prevalence of hog cholera during the past season may possibly be attributed to the excessive rains during the late spring and early summer months. Under such conditions, relative to rainfall, the hog-cholera virus may be washed from infected pens and yards into creeks and small streams, these tributaries emptying into rivers and flooded districts, thus sweeping the infection over large areas. In addition to the simple mechanical action involved in this manner of spreading the disease, the heavy rains during the past season served to keep up a high moisture content of the soil. The presence of plenty of moisture is, of course, favorable to the growth and multiplication of the lower organisms. Such a favorable condition, therefore, may have had considerable influence in spreading the disease and in maintaining the high degree of virulence of the material. Other means of dissemination, such as dogs, birds and boots of stock buyers, have doubtless contributed their share toward the wide distribution of the disease.

In 1885 Dr. Theobald Smith and Dr. D. E. Salmon isolated *Bacillus cholera suis* and described that organism as the specific cause of hog cholera. Since that time a continuous line of investigation has been conducted by the Bureau of Animal In-

dustry, United States Department of Agriculture, for the purpose of discovering some protective inoculation or treatment against the disease. During the past few years several investigators, both in Europe and America, have been actively engaged in this study. In 1903 the Bureau of Animal Industry published a preliminary report[1] in which it was stated that it was possible to transmit hog cholera to healthy swine by injecting the filtered serum of hogs suffering from cholera. Bacteriological examination proved the absence in this filtered material of *B. cholera suis* or other bacteria capable of growing on ordinary culture media. Since the appearance of the above report the investigations of the Bureau of Animal Industry, as well as the results of extensive work in other laboratories, have confirmed the belief that *Bacillus cholera suis* is not the specific cause of hog cholera, or, more cautiously stated, that *Bacillus cholera suis* is not the etiological factor in all forms of hog cholera.

It seems possible that *Bacillus cholera suis* may be a variety of the common intestinal parasite, *Bacillus coli communis*. The hog-cholera organism is pathogenic when injected in culture into experimental animals, including the hog; nevertheless, such is also true of *Bacillus coli communis*. Again, it may be possible that in those instances in which *Bacillus cholera suis* can be isolated from the liver, spleen and other tissues of hogs dead from cholera that the organism has appeared in those tissues, having migrated from the intestinal mucosa, as does the colon organism when it frequently appears in the spleen, liver, and tissues other than the intestinal mucosa, during certain processes of the disease. In other words, the presence of *B. cholera suis* in certain cases may indicate the result and not the cause of the disease.

Bacillus cholera suis is certainly not exclusively associated with the tissues of the diseased or of healthy hogs. White[2] isolated an organism culturally identical with the hog-cholera bacillus from the intestine of healthy honey-bees.

McClintock, Boxmeyer and Siffer[3] made a careful study of hog-cholera blood, relative to the presence of agglutinins for *Bacillus cholera suis*. In their work twenty-two specimens of

1. De Schweinitz and Dorset: Bureau of Animal Industry, 1903, circular 41.

2. White, G. F.: Bureau of Entomology, Technical Series No. 14, 1906.

3. McClintock, Boxmeyer and Siffer: Journal of Infectious Diseases, vol. II, No. 2, March 1, 1905.

normal hog blood and fifty-seven specimens of blood from cases of hog cholera were examined. They summarize the results obtained by the statement that "Agglutination is of no value for the diagnosis of hog cholera as the disease is at present defined." Hottinger,[4] in a recent article on "*Bacillus suipestifer*," in which he bases his observations upon certain comparative cultural and pathogenic characters of *B. cholera suis* and *B. coli communis*, concludes as follows: "From the foregoing observations, it would appear that *B. cholera suis* is a migratory colon-like intestinal organism, having acquired pathogenic properties, which is always, however not exclusively, found in cases of hog cholera." According to the present state of knowledge regarding the etiological factor involved in hog cholera, many investigators are led to believe that certain assumptions may be correct. Experimental evidence certainly indicates the presence of some living organism in hog-cholera virus.

II.

ATTEMPTED CULTIVATION OF FILTERED VIRUS OF HOG CHOLERA.

Collodium Sac Method.

The brilliant investigations of Nocard and Roux,[5] in employing the collodium sac as a means of artificially growing the virus of contagious pleuropneumonia in cattle, resulted in the identification of a very small, almost ultra-microscopic, motile organism. By inoculating cultures of these minute organisms, Nocard and Roux produced the disease in healthy cattle. The results of Nocard's work suggested the possible advantages of such a procedure with reference to the filterable hog-cholera virus. A series of experiments therefore were conducted in using the collodium-sac method as a possible means of artificially cultivating the virus of hog cholera. This work was begun in August, 1905, by Dr. C. H. Boxmeyer, and after September 1, 1905, was conducted by the writer.

Collodium sacs, prepared according to Novy,[6] were filled with hog-serum broth as the artificial culture medium. This medium was prepared by the addition of equal parts of normal

4. Hottinger, Robert: Centralblatt für Bakteriologie, etc. Orig. Bd. XLVII, July, 1908, p. 186.

5. Nocard: Handbuch d. Path. Micro-organismen, 1903, III, p. 682. Nocard and Roux: "Le Microbe de la peripneumonie." Récueil de med. Veterinaire, March 24, 1898, p. 213.

6. Novy, F. G.: Laboratory Work in Bacteriology, second ed., p. 499.

hog serum and ordinary nutrient broth. These media were inoculated with the hog-cholera virus or serum, and after inoculation were placed in the abdominal cavity of the rabbit, where they were allowed to remain for several days. At the time of each serial passage, check collodium sacs, containing the uninoculated media, were placed in the abdominal cavity of the rabbit. The first passage through the rabbit consisted of collodium sacs filled with equal parts of normal hog-serum broth and virulent hog-cholera serum from hog No. 147. This strain of virus originated from an outbreak at Pontiac, Mich. The serum from hog No. 147, before the inoculation of the collodium sac, was filtered through a Chamberland porcelain filter. The collodium sacs of the first passage were allowed to remain in the abdominal cavity of the rabbit for a period of twenty days. At the expiration of that time the four rabbits

TABLE 1.

COLLODIUM SAC PASSAGES.

Number generation.	Period of time allowed for each passage.	Number of rabbits used and diagram showing source of inoculations of each collodium sac.	Inoculations. Parts contents preceding sac to parts of normal hog-serum broth.	Dilution of original virus from Hog 147
		Hog 147		
1st	20 days	Rabbits 323 325 327 328	1 to 1	$\frac{1}{2}$
2nd	6 days	Rabbits 333 330 329	1 to 9	$\frac{1}{20}$
3rd	25 days	Rabbits 334 335 336 337	1 to 30	$\frac{1}{600}$
4th	16 days	Rabbits 343 344 339 345 342 346	1 to 50	$\frac{1}{3000}$
5th	10 days	Rabbits 347 348 349 353 350 352	1 to 30	$\frac{1}{90000}$
6th	10 days	Rabbits 351 357	1 to 20	$\frac{1}{1800000}$

which contained the inoculated and uninoculated (control) collodium sacs were anesthetized and the sacs removed.

After a careful bacteriological examination of the contents of the collodium sacs, transfers were made from the contents of the inoculated sacs of the first passage into a second series of normal hog-serum media, and the second series was subjected to a second passage through the rabbit. After this manner the original filtered virus from hog No. 147 was passed through six generations or passages in the abdominal cavity of the rabbit. Table 1 shows in condensed form the time duration for each passage, extent of dilution of the original hog-cholera virus, and the number of the rabbit used in each passage.

A very careful bacteriological examination was made of the contents of each collodium sac after its removal from the abdominal cavity of the rabbit. Several of the sacs (in rabbits 323, 328, 339, 348, 349, 350 and 353) were found to be contaminated and were not used for reinoculation. Contents of collodium capsules 334, 335 and 343, which were contaminated with micrococci, were filtered through a Pasteur filter, and from their filtrates, after bacteriological examination, sacs 343, 344, 339, 345 and 347 of succeeding generations were respectively inoculated.

In no instance could the presence of any growth be detected in the contents of the collodium capsules. The contents of each sac was subjected to the most careful microscopical examination. Various attempts were made to obtain growths by making transfers from the collodium-sac contents to coagulated normal hog-serum media, normal hog-serum bouillon and normal rabbit-serum bouillon. Experimental animals were inoculated with material from the capsules.

Frequently a slight cloudiness could be detected, which, upon comparison with the control sacs, was found not to denote the presence of any apparent growth. This cloudiness appeared to be caused by a precipitate which probably resulted from the hog serum. At the completion of the sixth passage a hog was injected with some of the contents of sacs from rabbits 351 and 357, with no apparent results.

III.

IMMUNIZATION AGAINST THE DISEASE.

The study of hog cholera in all its various phases centers in one intensely practical problem, namely, that of preventive inoculation. Before the recognition of the fact that *Bacillus cholera suis* was not the specific cause of the "filterable-virus form" of the disease, many ineffectual attempts were made to produce some protective substance, using the hog-cholera organism as the basis. Since the time that *Bacillus cholera suis* has been practically eliminated as the etiological factor of the disease, various attempts have been made to cultivate artificially the filterable virus. All of these attempts have produced negative results. The attention of many investigators therefore has been turned toward the production of a vaccine or antitoxin for the disease, using the filterable virus as a basis.

Attempts to attenuate the hog-cholera virus by the use of various chemicals and by drying at different temperatures have thus far met with failure.

In 1897 Kolle and Turner[7] published the results which attended their method of simultaneous vaccination in combating rinderpest in South Africa. The simultaneous method of vaccination of healthy hogs as a protection against hog cholera has been the subject of an extensive line of experimental work by the Bureau of Animal Industry, United States Department of Agriculture. As a result of their work the Bureau of Animal Industry has recently developed the method to such an extent that several state experiment stations are making preparations to manufacture the vaccine and supply the product at cost to swine owners. The essential features of this method consist in the subcutaneous, simultaneous injection of hyper-immune serum and disease-producing blood into the healthy hog. In other words, the healthy animal is inoculated with from 1 to 3 cc. of hog-cholera virus and at the same time receives from 10 to 30 cc. of serum from a hog which has been hyper-immunized against the disease. Apparently the reaction which follows the inoculation with the virus is inhibited by the influence of the hyper-immune serum and the vaccinated animal consequently acquires a sufficient degree of immunity to protect it against subsequent exposures to hog chol-

7. Kolle and Turner: Deutsch Med. Wochnsch, 1897, p. 793.

era. The duration of immunity extends over a period of several months. The hyper-immune serum is obtained from hogs which are first immunized either through natural or acquired immunity, and later are subjected to hypodermic injections of large quantities of hog-cholera blood serum.

There is no question as to the efficiency of the simultaneous method. Extensive successful experimentation by the Bureau of Animal Industry, as well as confirmatory experimental evidence obtained by other workers, shows beyond a doubt that the above procedure successfully vaccinates. The United States Department of Agriculture is to be commended for its persistent and effective work upon hog cholera. Much credit is due to those connected with the Bureau of Animal Industry for their determined and intelligent scientific efforts to eradicate this disease. From a practical point of view, however, "Dorset's Hog Cholera Vaccine," or the simultaneous method of vaccination, has certain important disadvantages. "Dorset's Hog Cholera Vaccine" is not, at present, practical, because of the expense involved in its preparation. The fundamental reason for this expense is the fact that a hog yields only a small amount of blood in comparison with most other animals. At the beginning of the preparatory procedure, hogs are immunized, either naturally or artificially, against the disease. It is presumable that during this process some animals may be lost. With the next step the immune hogs are hyper-immunized by the injection of relatively large quantities of hog-cholera serum. This serum must be procured from a large number of hogs suffering from the acute form of the disease. These animals may be located at the vaccine plant itself, or in the field on various infected farms. The third and last essential step is the obtainment of the blood serum from the hyper-immunized animals. This is the most disappointing part of the whole procedure. Whether the hyper-immune animal is relieved at one time of all its blood from the carotid artery or whether it is required to yield its body fluid by several instalments from the tail, the results are not highly encouraging, because of the relatively small amount of blood which can be secured. This fact, taken in connection with the experimental evidence that ten or more cubic centimeters of the immune serum are necessary in the preventive treatment of a fifty-pound pig, presents serious difficulties to those who may wish to apply the method in a practical way.

Again, the simultaneous method of vaccination may not be of advantage because of the danger accompanying the use of the necessary pathogenic hog-cholera virus as a part of the vaccination. There is always more or less risk involved in distributing various vaccines. It seems reasonable to suppose that, in comparison with attenuated products such as black-leg vaccine, there would be much more risk concerned in placing upon the market a hog-cholera vaccine, one part of which is virulent hog-cholera serum. The danger that might follow the broadcast distribution of hog-cholera virus, in some instances among careless veterinarians and uninformed farmers, cannot be ignored.

IV.

ATTEMPTED ATTENUATION OF HOG-CHOLERA VIRUS BY PASSAGE THROUGH ANIMALS OTHER THAN THE HOG.

Various unsuccessful attempts have been made to communicate the disease of hog cholera to animals other than the hog. Repeated inoculations have shown that the common laboratory animals are not susceptible to the filterable virus.

In 1905 a number of series of experiments were undertaken by the writer in an attempt to determine whether hog-cholera serum could be attenuated or modified by passage through some domesticated animal. The sheep was the first animal upon which any extensive work was conducted. About fifteen sheep were used. Most of the animals were injected in the jugular vein with hog-cholera serum, the average amount injected being about 100 cc. The sheep were bled at varying intervals of time and their serums introduced into healthy hogs. Several days after treatment with the sheep serum, the hogs, together with controls, were exposed to hog cholera. This work was attended with variable results, some negative, some positive, all, on the whole, distinctly lacking in uniformity. The general conclusion was reached that under some conditions passage through the sheep attenuated the virus in such a way that it would successfully vaccinate healthy hogs. It appeared, however, that no definite degree of uniformity or standardization of the experimental sheep-serum vaccine could be obtained. The employment of the sheep was therefore suspended, and the next animal used was the donkey.

The results obtained in the experimental work with the donkey-serum vaccine were more encouraging. On the whole,

these experiments were quite uniform. One of these series appears in the following pages. After some work with the donkey, the horse was used as a medium in attempting to produce a satisfactory vaccine. The horse was substituted for the donkey because of the general adaptability of the horse to such work and the comparatively large yield of blood which may be obtained from this animal. Uhlenhuth, Hubener, Xylander and Bohtz,[8] in 1908, stated that "the serum of the horse and donkey which were treated with repeated intravenous injections of virus containing fluids had neither a protective nor a healing property. Whether the hyper-immunization of the animal will enhance the production of anti-bodies must be determined."

No attempt has been made in this work to produce an experimental hog-cholera antitoxin from the horse. The work has been directed more particularly toward the attempted production of a horse-serum hog-cholera vaccine.

V.

POSSIBLE TOXIC ACTION OF HOG-CHOLERA SERUM ON THE HEALTHY HORSE.

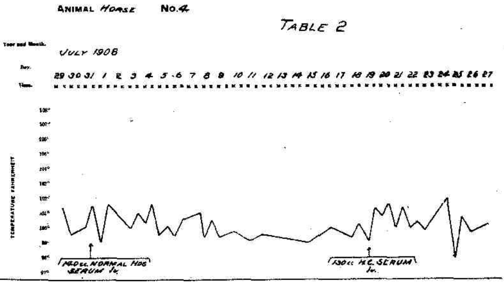
Repeated observations at the Kansas Experiment Station have shown that the intravenous injection of hog-cholera serum into the horse produces in that animal immediate and well-marked symptoms. The most pronounced symptoms are accelerated pulse, followed by irregular and weakened heart action, quickened respiratory functions, and violent peristalsis. The writer has found that the average horse cannot receive at one time more than 150 to 200 cc. of virulent hog-cholera serum. Experiments indicate that an intravenous dose in excess of from 150 to 200 cc. will invariably cause very serious disturbances and frequently death. The symptoms appear soon after the injection of the virus and continue from one to six hours. Not until comparatively recently in the course of this investigation has much attention been paid to the possible toxic action of hog-cholera virus upon the horse. The serious disturbances caused by such injections were carefully noted, but the impression was held that the symptoms were produced simply by the direct introduction of foreign serum into the

8. Uhlenhuth, Hubener, Xylander, Bohtz: "Untersuchungen über das Wesen und die Bekampfung der Schweinepest," Arb. A. d. Kaiserl. Gesundheitsamt, Bd. 27, 1908, H. 3.

circulatory system of the animal. The following appended notes show some of the observations which have been made and which suggest the toxicity of hog-cholera serum for the horse when injected intravenously:

Horse No. 4, bay gelding, weight about 1300 pounds, in good condition, except lameness in right front limb due to contracted tendon. July 31, 1908, four P. M., horse No. 4 was injected in jugular vein with 140 cc. normal hog serum. Fifteen minutes after injection heart action and respiration slightly accelerated. One hour after injection temperature was 103 degrees. One-half hour after treatment a few raised blotches or pimples appeared on the skin. August 1, eight A. M., temperature normal and skin eruptions had disappeared.

August 19, 9:25 A. M., same horse (No. 4) injected with 130 cc. of Norwood, Kan., strain, filtered hog-cholera serum. Immediately after treatment the respiration and heart action were greatly accelerated, both rapidly increased for twenty minutes. One-half hour after injection heart became depressed and pulse almost obliterated. At this time the animal broke out in a profuse, cold, clammy sweat. At the end of forty-five minutes the horse became very weak, staggered and fell. Eye dull and listless. During this time peristalsis was very violent, within twenty minutes after treatment the animal having had ten profuse watery evacuations. The effect in this particular was like that from a dose of eserin. One-half hour after injection the animal received orally four ounces of alcohol, and forty-five minutes afterwards one-fourth grain of digitalin was injected subcutaneously. After two hours the animal began to slowly recover, and by evening appeared normal with the exception of a condition of extreme exhaustion. The temperature of horse No. 4 during the above treatment appears in the accompanying chart (table 2):



The results of the above experiment were far from conclusive, in the first place, because of the possibility of some

reaction allied to anaphylaxis. Rosenau and Anderson[9] have called attention to such a condition of sensitization which is produced by the introduction of repeated injections of horse serum into the guinea-pig. It perhaps might be suspected, therefore, that horse No. 4, which had received an intravenous injection of normal hog serum twenty days before the intravenous injection of hog serum, had become sensitized to the hog serum to such an extent that the marked symptoms following the second injection were those pertaining to some reaction resembling anaphylaxis.

In order that this point relative to a possible anaphylactic reaction might be made more clear, a second horse was injected in the jugular vein, at intervals on the average of every seven days, with increasingly larger doses of hog-cholera serum. The animal received seven treatments after this manner. The horse received the seventh intravenous injection of hog-cholera serum, consisting of 75 cc., on August 11, 1908. Eleven days afterward the animal was injected with normal hog serum. The following observations and appended temperature chart give the data on this experiment:

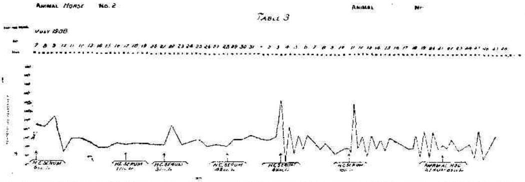
Horse No. 2, gray gelding, in fair condition. This animal had received the following injections, intravenously, of hog-cholera serum (Norwood strain): July 4, 3 cc.; July 7, 6 cc.; July 17, 17 cc.; July 21, 30 cc.; July 28, 42 cc.; August 3, 68 cc.

On August 11, 1908, 10 A. M., horse No. 2 was injected with 75 cc. of hog-cholera serum (Norwood, Kan., strain). Five minutes after treatment the animal broke out in a copious, cold, clammy perspiration. The respiration was painfully accelerated and heart action rapid, at times very irregular. The heart finally became weak and the pulse was almost obliterated. Peristalsis very active and severe purging and watery evacuations followed. These symptoms extended in grave form over a period of about three hours. No stimulants were administered, and after three and one-half hours the animal gradually became more comfortable. Six hours after the injection the respiratory functions were nearly normal although the heart was still weak and the animal was greatly exhausted. Seven hours after the treatment the temperature was nearly 104 degrees. (See table 3, page 49.)

On August 22 the same animal, horse No. 2, received intravenously 85 cc. of normal hog serum. After treatment the animal showed slightly accelerated pulse and respiration. These disturbances were detected by careful examination. Five minutes after injection the horse was turned out in paddock and immediately started eating grass. The animal was

9. Resenau and Anderson: "A Study of the Cause of Sudden Death Following the Injection of Horse Serum" and "Further Studies upon Anaphylaxis," Bulletins 29 and 45, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv., Wash.

carefully watched for some time. However, no symptoms other than very slight disturbances of the heart and respiration were noted.



It will be noticed in both experiments indicated above that in each instance the animal received of the normal hog serum 10 cc. in excess of the amount of hog-cholera serum injected. In both instances the hog-cholera virus, in comparison with the normal hog serum, produced marked symptoms which strongly suggest the toxicity of the cholera virus upon the horse.

VI.

MODIFIED CONDITION OF VIRULENT HOG-CHOLERA SERUM AFTER SHORTER OR LONGER RESIDENCE IN THE BLOOD OF THE DONKEY AND HORSE.

In the experimental use of the horse as a medium for the modification of the hog-cholera virus the work logically resolves itself into the solution of certain definite points. Fundamentally, the subsequent experiments depend upon the proof of the fact that a residence in the blood of the horse causes attenuative changes to take place in the hog-cholera virus. If the virus does become attenuated, experiments must show whether the modifications of the virus are reasonably uniform and under what conditions the attenuation takes place. More specifically, a determination must be made of the exact period of residence in the blood of the horse which will cause the proper degree of attenuation, so that the recovered modified hog-cholera virus will act as a vaccine and not as a disease-producing agent.

In carrying out these experiments the horse is injected in the jugular vein with a given quantity of virulent hog-cholera

serum. The amount of virus introduced intravenously, depending upon the animal used, varies from 85 to 200 cc.

The horse is then bled at various intervals of time after the injection. The blood is placed in a refrigerator, and after a few days the equine serum is collected and tested upon healthy swine.

1. *Strain of filterable hog-cholera virus used in present investigation.*

The strain of hog-cholera virus which is now employed in the present investigation was obtained from an outbreak at Norwood, Kan. This material represents a very acute type of the disease and is well adapted to use in experimental work. The history of this outbreak is as follows:

On July 14, 1908, the farm of Clinton Eversol, one-half mile west of station of Norwood, was visited. It was found that acute hog cholera existed on this farm. Three weeks before the time of visit, Mr. Eversol had a herd of 200 hogs. On July 14, he probably had left about one-third of the original herd of 200. The disease affected the young pigs in a more acute manner than the older hogs. There were probably 40 or 50 spring pigs, all of which showed symptoms of the disease in various stages. Some of the sows were sick and several of them had died. A pig weighing perhaps seventy-five or eighty pounds, which had showed typical symptoms of hog cholera for a few days, was bled aseptically from the cartoid artery. After bleeding a careful *post-mortem* was made, and typical lesions of severe hog cholera were found. The skin of the ventral portion of the body was discolored; the ears were purplish; subcutaneous tissues contained blood extravasation; portions of the dependent lobes of the lungs were hepatized; heart apparently normal; liver normal; kidneys showed a few petechiæ. The spleen was very much enlarged and gorged with blood. The cæcum and large intestine showed the presence of very typical ulcers. The spleen and specimens of the intestinal ulcers were brought to the laboratory and preserved as museum specimens.

Origin of the Outbreak.—According to the statements of Mr. Eversol, an epizootic of hog cholera existed last fall and winter in that county and north of it. During the very rainy season last spring and early summer his hogs had access to the overflow of water from a creek which runs through the farm.

It is believed that the virus was conveyed by the creek, coming down from the infected farms north of it. As evidence of this origin of the outbreak, it was found that four other farms in the immediate locality were infected with hog cholera, and that the hogs belonging on these farms had had access to the creek water. Several farms in the neighborhood bordering on this creek were free from cholera, but the hogs on these did not have access to the creek water.

2. *Virulent character of the blood serum of the horse, two to seven hours*[10] *after intravenous injection of the animal with hog-cholera virus.*

Experimental evidence indicates that a portion of blood secured from the horse two to four hours after the animal has received 85 to 200 cc. of hog-cholera serum intravenously will manifest virulent properties when injected intravenously into the healthy hog. The following data relate to this phase of the work:

On July 17, 1908, 11 A. M., horse No. 3, bay gelding in good condition, was injected intravenously with 150 cc. of hog-cholera serum (Norwood strain). The usual symptoms as described above were exhibited. Heart at first greatly accelerated, later depressed; respiration accelerated and difficult; peristalsis violently active. The temperature of the animal four hours after the injection was 103 degrees. Four hours after the injection two liters of blood were drawn, and twenty-four hours after five liters were obtained.

On July 18, 1908, hog No. 9, small red Duroc-Jersey, weight 30 pounds, was injected in the ear vein with 5 cc. of horse No. 3, 4-hour serum. On July 23, five days after inoculation, this pig showed loss of appetite, languor, and thermal reaction of 105.8 degrees. (See table 4.) After the fifth day the animal refused all food, remained in a corner, was constipated and became greatly emaciated. On the fourteenth day after inoculation, August 1, the pig being moribund, was chloroformed, bled from the carotid artery, and autopsy made. Typical lesions of hog cholera were found. On the ventral portion of the body were purple discolorations of the skin; blood extravasations in the subcutaneous tissue; portion of the dependent lobes of both lungs were congested and hepatized; spleen soft and gorged with blood; liver and kidneys normal; the inguinal, mediastinal and mesenteric lymphatic glands enlarged and hemorrhagic. Evidences of ulceration were present in the cæcum. (See plate 6.)

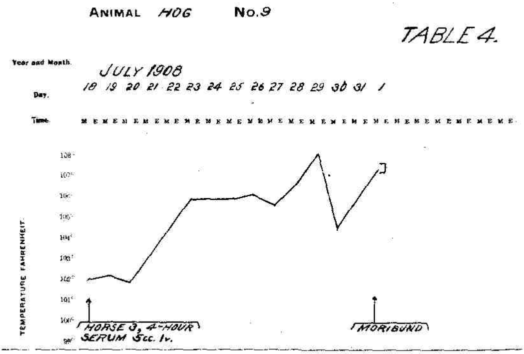
As a means of control or check on the above experiment

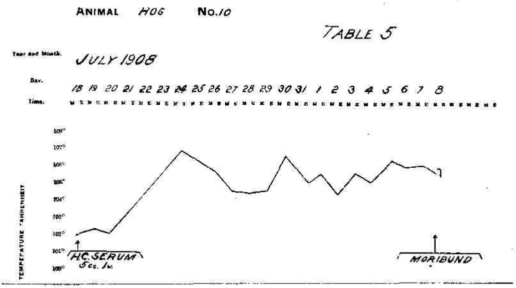
10. Hereafter, in this bulletin, "2-hour horse serum," "4-hour horse serum" or "24-hour horse serum" will be used to indicate that the serum in question was obtained from the horse two hours, four hours or twenty-four hours after the animal was injected intravenously with hog-cholera serum.

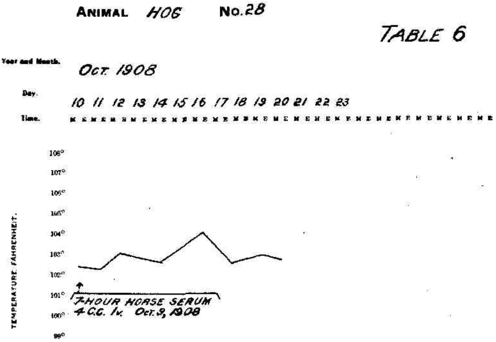
with the 4-hour horse serum, the following inoculation experiment was conducted with the original Norwood hog-cholera serum:

Hog No. 10, belonging to the same litter with hog No. 9, on July 18, 1908, was injected in the ear vein with 5 cc. of the same lot of hog-cholera serum which was used in the preparation of the 4-hour horse serum, horse No. 3. Five days after inoculation, July 23, this animal manifested loss of appetite and constipation. The usual symptoms became gradually more pronounced for the next few days, but by August 30 the appetite had returned and the pig became brighter. On August 5, however, typical symptoms of acute cholera were again present, and on August 8, twenty-one days after inoculation, the animal, being moribund, was chloroformed, bled, and autopsy performed. (See table 5.) Typical lesions of cholera were found, although not as severe pathological conditions were present in proportion to the duration of infection in each case as those which were found in hog No. 9.

TABLE 4.

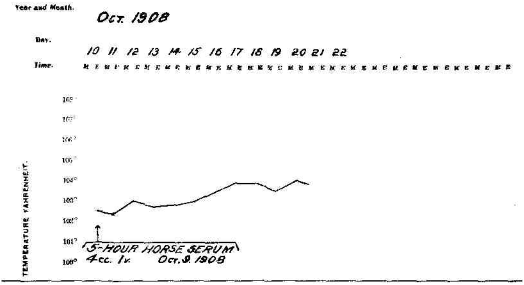



ANIMAL HOG No. 10
TABLE 5
Year and Month. JULY 1908
Day. 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8
Time.
TEMPERATURE FAHRENHEIT
H.C. SERUM 5cc. Iv.
MORIBUND


ANIMAL HOG No. 28
TABLE 6
Year and Month. OCT. 1908
Day. 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Time.
108° 107° 106° 105° 104° 103° 102° 101° 100° 99°
TEMPERATURE FAHRENHEIT.
7-HOUR HORSE SERUM 4 C.C. Iv. OCT. 9, 1908

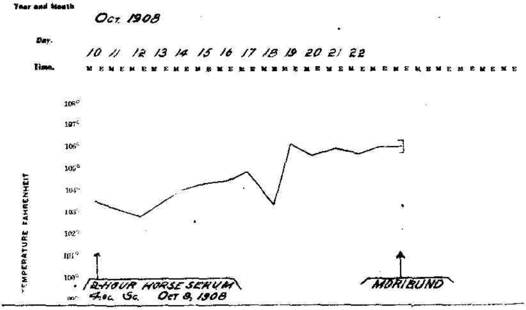
ANIMAL *HOG* No. 29

TABLE 7.



ANIMAL *HOG* No. 30

TABLE 8



On October 9, 1908, hogs Nos. 28, 29 and 30 were injected subcutaneously with 2- to 7-hour horse serum. The results of this test indicate the virulent nature of 2-hour horse serum. This experiment also logically shows that attenuative changes in the hog-cholera virus take place after a residence of from five to seven hours in the blood of the horse.

The temperature curves of hogs Nos. 28, 29 and 30 are shown in tables 6, 7 and 8. The following appended notes give the details of the experiment:

Hogs Nos. 28, 29 and 30, weight about 30 pounds each, all belonging to the same litter, on October 9, 1908, were treated as follows: Hog No. 28, injected intravenously with 4 cc. 7-hour horse No. 3 serum; hog No. 29, injected intravenously with 4 cc. 5-hour horse No. 3 serum; hog No. 30, injected intravenously with 4 cc. 2-hour horse No. 3 serum.

Hog No. 28, seven days after inoculation with 7-hour horse serum, showed a temperature reaction of 1½ degrees. (See table 6.) No other deviations from normal conditions occurred and the animal is at present in healthy condition.

Hog No. 29, seven days after receiving the 5-hour horse serum, showed a slight rise in temperature (see table 7), which continued for several days. On October 19, nine days after inoculation, hog No. 29 appeared to have a slight chill and showed loss of appetite. By August 21, twelve days after inoculation, the animal was normal in every way and is at present in good condition.

Hog No. 30, on October 14, five days after inoculation, had a temperature of 104 degrees, which gradually rose on October 17 to 104.8 degrees. (See table 8.) The animal showed loss of appetite, constipation, and the usual symptoms of hog cholera. On October 19, the temperature, after having dropped to 103.4 degrees on the preceding day, reached 106 degrees. The symptoms steadily became more pronounced, and on October 24, fifteen days after inoculation, hog No. 30 being moribund, was chloroformed, bled, and autopsy performed. The lesions were those of the acute type of hog cholera.

When one considers that the hog-cholera virus is introduced into the horse in the rough proportion of 150 cc. of virus to 30,000 cc. or more of horse blood, making a dilution of 1 part of hog-cholera virus to 200 or more parts of horse blood, and that the 2- to 4-hour horse serum will produce the typical form of the disease in a healthy hog, it may be theorized that for the first few hours after the injection of the animal, the hog-cholera virus or organism rapidly multiplies in the body fluid of the horse. The results obtained from inoculation of hogs Nos. 28 and 29 with 7- and 5-hour horse serum, respectively, make evident the fact that after five to seven hours' residence in the blood of the horse the hog-cholera virus undergoes at-

tenuative changes. In the following pages it will be seen from a number of series of experiments that 24-hour horse serum is more attenuated in virulence. Therefore we may assume that this attenuation is not due to mere dilution of the hog-cholera virus in the horse's blood, but that it is the result of physiological activity and antagonistic forces; a process of attenuation which should be found to be uniform and constant to a greater or lesser degree.

The above experiment is of considerable importance in that it tends to prove that 2- to 4-hour horse serum contains the virus in sufficient quantity and strength to produce the disease in the acute form. This experimental evidence may be of some aid in determining the nature of the organism which is the specific cause of the disease.

3. *Intravenous versus subcutaneous inoculation with 4-hour horse-serum hog-cholera virus.*

The above experiments indicate the infectious nature of 4-hour horse serum when injected intravenously into the healthy hog. An attempt was then made to produce the disease by injecting 4-hour horse serum subcutaneously. On September 2, 1908, hog No. 22 was injected subcutaneously with 4 cc. of horse No. 4, 4-hour serum. On September 4, 1908, hog No. 23 received subcutaneously 4 cc. of horse No. 3, 4-hour serum. No apparent results followed the above inoculations, except a possible temperature reaction in the case of hog No. 23 on the fifth day after inoculation. (See tables 37 and 38.)

On September 15, hogs Nos. 22 and 23 were each injected intravenously with 5 cc. of the same respective lots of 4-hour horse serum which they had previously received subcutaneously. On the same date, hog No. 26, a control, was injected in the ear vein with 5 cc. of horse No. 3, 4-hour serum. Hogs Nos. 22 and 23 remained in a normal condition, while hog No. 26, the control, showed the usual symptoms of the disease after an incubation period of seven days, and became moribund on the twelfth day after inoculation.

Thus it appears from this preliminary work that 4-hour horse serum contains highly virulent properties when injected intravenously into the healthy hog, while the subcutaneous injection of the same does not produce the disease. Furthermore, the subcutaneous inoculation of the 4-hour horse serum is not inert, because it shows protective properties. (See plate 13.)

This phase of the work is far from being completed. Determinations relative to pathogenetic and protective properties must be made of horse serum drawn at close intervals of time from one-half hour to several hours after the animal has received the hog-cholera virus in the blood. It is obvious that this work is of considerable practical value.

The thought suggests itself that, in case a satisfactory vaccine cannot be obtained from horse serum, this part of the method might possibly be utilized in the simultaneous method. Assuming that conclusive investigations should prove that 2- to 4-hour horse serum maintains a constant virulent character, then this horse serum virus might be substituted for the hog-cholera serum virus in the preparation of "Dorset's Hog Cholera Vaccine." Such a substitution would tend to reduce the expense of the simultaneous method.

4. *Attenuated character of hog-cholera virus after twenty-four hours or more residence in the blood of the horse.*

The following data, in comparison with the intravenous injection of 4-hour horse serum, show the results obtained by the same method of inoculation when 24-hour horse serum is employed:

Hog No. 245, weight 60 pounds, on February 26, 1907, was injected in the ear vein with 10 cc. of 24-hour horse serum. (Gray gelding, blood drawn twenty-four hours after being injected in the jugular vein with 150 cc. of mixed hog-cholera serum.) Hog No. 245 received subcutaneously at the same time 10 cc. of the same virus. On March 4 this animal received intravenously a second injection of the same lot of 24-hour horse serum. Five days after the second injection the animal showed a marked thermal reaction, the temperature remaining between 105 degrees and 106 degrees for four days, after which it became normal. This pig showed no loss of appetite or other symptoms at any time during the treatment. On April 15 he weighed 82 pounds, showing a gain of 22 pounds since the beginning of the treatment on February 26.

Hog No. 247, weight 61 pounds on February 26, 1907, received 10 cc. intravenously and 10 cc. subcutaneously, of 24-hour horse serum. On March 4 he was injected the second time intravenously with 24-hour horse serum. This animal showed no disturbance in temperature and exhibited no symptoms whatever as a result of the treatment; during the first eleven days of the treatment he gained 2½ pounds.

Hog No. 248, weight 46½ pounds, on February 26, 1907, received intraperitoneally 10 cc. and subcutaneously 10 cc. of 24-hour horse serum. Seven days afterward he received intravenously 10 cc. more of the same material. During the eleven days of treatment the animal gained 7¼ pounds in weight. He showed no untoward symptoms as a result of the

inoculations, although later the animal died of acute hog cholera after natural exposure.

The temperature charts of hogs Nos. 245, 247 and 248 appear in tables 22, 24 and 25.

The statement should be made that these experiments are not conclusive proof that 2-hour horse serum injected subcutaneously in small quantities and 4-hour horse serum injected intravenously always produce the disease in the healthy hog, and that 5- to 7-hour horse serum subcutaneously and 24-hour horse serum intravenously injected never cause infection. The great variation among individual animals, and the peculiar idiosyncrasies of swine particularly, preclude such a definite assertion.

5. *Protective properties of experimental horse-serum hog-cholera vaccine.*

The production of a satisfactory hog-cholera vaccine constitutes the practical problem which is included in this investigation. The attempt is being made to produce such a vaccine by passing the hog-cholera virus through the horse. A vaccine prepared according to such a method, providing it were efficient, would prove to be highly practical because of its comparative inexpense. A rough estimate of the actual cost of horse-serum vaccine will readily show that it can be prepared at a nominal expense. A very liberal estimation would place its cost at from one-third to one-half of that involved in the manufacture of the vaccine used in the simultaneous method.

The results so far obtained are strictly those from laboratory and experimental work and are not sufficiently confirmed to warrant the assumption that they are definitely conclusive. The experiments must be verified by repeated observations of the same under various conditions before definite deductions can be drawn.

The results of the following series of experiments tend to demonstrate that under certain conditions healthy hogs can be successfully vaccinated by the use of the method.

On January 18, 1907, hogs Nos. 235, 236 and 237 were treated as follows:

Hog No. 235 was injected with 10 cc. intravenously and 10 cc. subcutaneously of 24-hour donkey serum; hog No. 236, 24-hour donkey serum, 10 cc. intravenously, and 48-hour donkey serum, 10 cc. subcutaneously; hog No. 237, 48-hour donkey serum, 10 cc. subcutaneously, and 72-hour

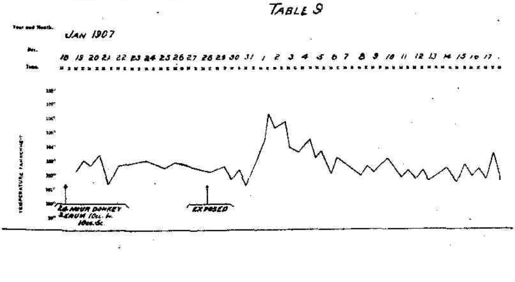
donkey serum, 10 cc. subcutaneously. In none of these hogs was any apparent reaction noted as a result of the vaccination.

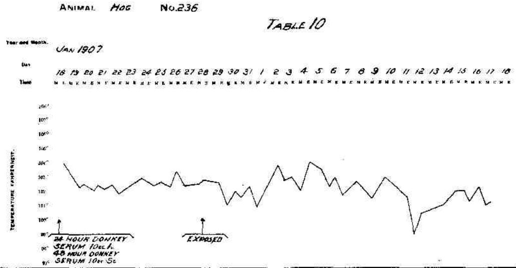
On January 28, ten days after vaccination, hogs Nos. 235, 236 and 237, together with hog No. 238, an untreated control animal, were exposed to the disease by being placed in an infected pen and by being fed viscera from hog No. 234. Hog No. 234 was chloroformed, bled and posted on January 22, having had an acute case of the disease of twelve days' duration.

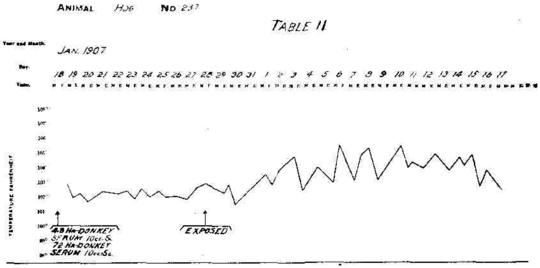
Of this series of four hogs, the control animal, No. 238, after five days' incubation period, exhibited severe symptoms of the disease. On the fifth day the temperature rose to 107. (See table 12.) The animal refused to eat and became constipated and emaciated. On the eighth day after exposure this hog, being moribund, was chloroformed, bled and posted. The head, ears and ventral portion of the body showed a diffuse pink color. Autopsy showed blood extravasations in the subcutaneous and muscular tissues, lymphatic glands enlarged and hemorrhagic, kidneys ecchymotic and petechied. Several small ulcers were present in the cæcum and colon. Vaccinated hog No. 235, which belonged to the same litter with hog No. 238, on the fifth day after exposure showed a rise of temperature to 106 degrees. (See table 9.) This animal, however, did not suffer loss of appetite or show any symptoms of disease, other than a thermal reaction. After four days the temperature became normal. Twenty-two days after vaccination the animal was temporarily released.

Hog No. 236 showed no temperature reaction after exposure, and remained throughout in a normal condition. (See table 10.) Twenty-two days after exposure the animal was temporarily released.

Hog No. 237, which received the 48- and 72-hour donkey serum, on the eighth day after exposure showed a temperature of 105.2 degrees. (See table 11.) On the twelfth day after exposure symptoms of the disease appeared. On the twenty-first day after date of exposure this animal was found dead. Autopsy showed lesions of hog cholera less severe in type than those found in hog No. 238. The results of the above experiment appear in tabulated form in table 13.




ANIMAL HOG No.236
TABLE 10
Year and Month. JAN 1907
Day
Time
TEMPERATURE FAHRENHEIT.
24 HOUR DONKEY SERUM 10cc A.
48 HOUR DONKEY SERUM 10cc Sc
EXPOSED


ANIMAL HOG NO 237
TABLE 11
Year and Month. JAN. 1907
Day.
Time.
TEMPERATURE FAHRENHEIT
48 Hr DONKEY SERUM 10cc S.
72 Hr DONKEY SERUM 10cc Sc.
EXPOSED

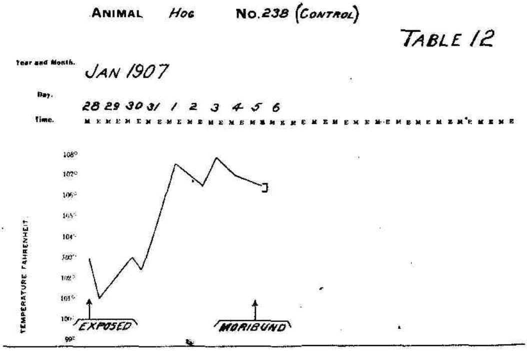

ANIMAL HOG NO.238 (CONTROL)
TABLE 12
Year and Month. JAN 1907
Day. 28 29 30 31 1 2 3 4 5 6
Time.
TEMPERATURE FAHRENHEIT.
EXPOSED
MORIBUND

TABLE 13.

EXPERIMENTAL VACCINATION.

Hog No.	Character of vaccine injected.	Method of vaccination.	Date of vaccination.	Period of time between vaccination and exposure.	Result of exposure.
235	24-hr. donkey serum 24-hr. donkey serum	10 cc. Iv.* 10 cc. Sc.*	Jan. 18, 1907 Jan. 18, 1907	10 days	Did not take disease.
236	24-hr. donkey serum 48-hr. donkey serum	10 cc. Iv. 10 cc. Sc.	Jan. 18, 1907 Jan. 18, 1907	10 days	Did not take disease.
237	48-hr. donkey serum 72-hr. donkey serum	10 cc. Sc. 10 cc. Sc.	Jan. 18, 1907 Jan. 18, 1907	10 days	Found dead from hog cholera 21 days after exposure.
238	Control	....	....	....	Moribund; bled and posted 10 days after exposure.

* Iv. = Intravenously; Sc. = Subcutaneously.

These hogs were exposed January 28, 1907, by being fed viscera of cholera hog No. 234.

From this series, the suggestion follows that hogs Nos. 235 and 236, which received the 24-hour donkey-serum vaccine, were protected against the disease by the vaccination. Additional proof of this is found in the fact that hog No. 235 showed some result of exposure coincident with the incubation period exhibited in hog No. 238. Hog No. 235 was therefore in all probability protected, not by natural, but by acquired, immunity. The successful vaccination of hog No. 236, which received the same treatment as hog No. 235, furnishes additional proof of the positive result. The value of the experiment is also logically strengthened by the presence of a less severe type of the disease in hog No. 237, which received subcutaneously 48-hour donkey-serum vaccine, as compared with that which appeared in the control, hog No. 238.

The results following the treatment of another series of eight hogs with horse-serum vaccine are shown in table 14.

Attention should be called to the fact that hogs Nos. 241 and 242 both died before or at the time of exposure. In hog No. 241, which died before exposure, no lesions could be found. Some symptoms of rheumatism were noted, however, while the animal was under treatment. Hog No. 242 was found dead the next morning after eating the infected viscera. On autopsy no lesions were found, except a pronounced congested condition of the mucous membrane of the alimentary tract. Some of the viscera which were fed had been kept in the ice-chest for several weeks and it might appear that hog No. 242 died from ptomain poisoning.

TABLE 14.

EXPERIMENTAL VACCINATION.

Hog No.	Vaccine injections	First injection. Material injected; date.	Second injection. Material injected; date.	Third injection. Material injected; date.	Time between last injection and exposure.	Result.
239	3	February 18. 24-hr. horse serum, 10 cc. Iv. and Sc.*	February 26. 24-hr. horse serum, 10 cc. Ip.	March 4. 24-hr. horse serum, 10 cc. Iv.	12 days	Did not take the disease.
240	3	February 18. 24-hr. donkey serum, 20 cc. Sc.	February 26. 24-hr. horse serum, 20 cc. Iv. and Sc.	March 4. 24-hr. horse serum, 5 cc. Iv.	12 days	Did not take the disease.
241	2	February 26. 24-hr. horse serum, 20 cc. Ip.† and Sc.	March 4. 24-hr. horse serum, 20 cc. Iv. and Sc.			Found dead March 16, before exposure.
242	2	February 26. 24-hr. horse serum, 10 cc. Iv. and Sc.	March 4. 24-hr. horse serum, 20 cc. Iv.			Found dead March 13.
243	Control; no treatment.					Moribund. Bled and posted March 30.
244	2	February 26. 24-hr. horse serum, 20 cc. Sc. and Ip.	March 4. 24-hr. horse serum, 10 cc. Iv.		12 days	Did not take disease.
245	2	February 24. 24-hr. horse serum, 20 cc. Sc. and Iv.	March 4. 24-hr. horse serum, 10 cc. Iv.		12 days	Did not take disease.
246	Control; no treatment.					Moribund, March 26. Bled and posted.
247	2	February 24. 24-hr. horse serum, 20 cc. Iv. and Sc.	March 4. 24-hr. horse serum, 10 cc. Iv.		12 days	Did not take disease.
248	2	February 24. 24-hr. horse serum, 20 cc. Ip. and Sc.	March 4. 24-hr. horse serum, 10 cc. Iv.		12 days	Found dead March 30.

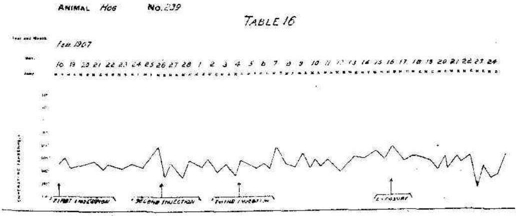
*Iv.=Intravenously; Sc.=Subcutaneously. †Ip.=Intraperitoneally.
These hogs were exposed March 16, 1907, by being fed viscera from hogs dead from cholera, and by being placed in an infected pen.

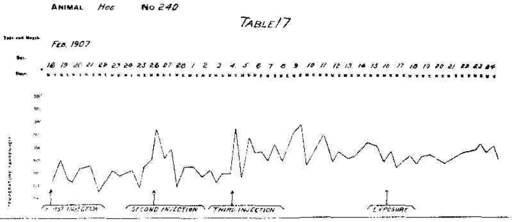
With the exception of hogs Nos. 241 and 242, both of which apparently died from other causes than hog cholera, all of the vaccinated hogs, except hog No. 248, withstood infection. This hog was evidently not protected by the vaccination. In this connection it should be noted that hog No. 244, while exhibiting a marked degree of immunization, did not gain in weight during and after exposure, as did most of the others. Hogs Nos. 243 and 246, the two control animals, both contracted the disease and were moribund at the end of fourteen and ten days, respectively, after exposure. In this experiment practically 83⅓ per cent. of the vaccinated hogs withstood infection. The weight and general condition of each of the animals in the above experiment at various times is given in table 15:

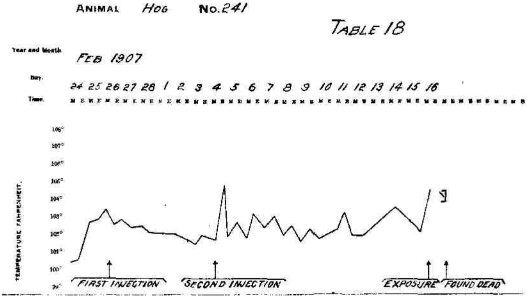
TABLE 15.

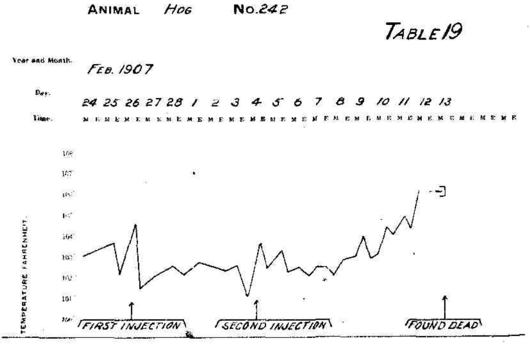
Hog No.	Vaccination treatment—No. of injections.	Weight March 1. *lbs.*	Weight March 7. *lbs.*	Weight and general condition April 15.
239	3	75½	87	90 lbs. In good condition.
240	3	26½	31⅛	Weight not recorded. In good condition.
241	2	38	50	Dead (not cholera).
242	2	46	54	Dead (not cholera).
243	Control	27½	34½	Dead (cholera). March 30.
244	2	29	33½	33 lbs. Eating well, but not gaining in weight.
245	2	60	68	90 lbs. In fine condition.
246	Control	....	....	Dead (cholera), March 26.
247	2	61	69½	Weight not recorded. In good condition.
248	2	46½	53	Dead (cholera).

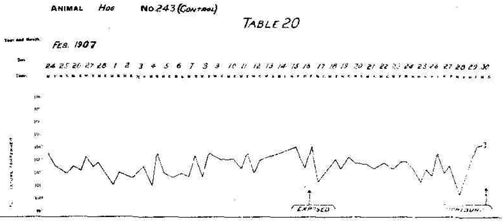
The temperatures of the above hogs during protective inoculations and subsequent exposure are shown in tables 16 to 25:

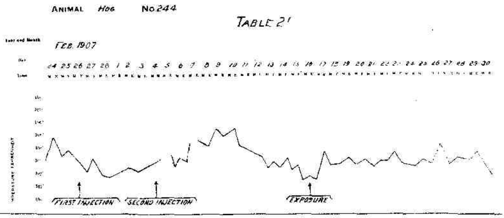


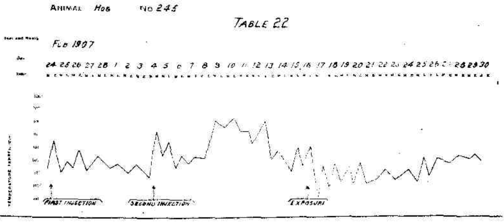

ANIMAL Hog No 240
TABLE 17
Feb. 1907
FIRST INJECTION
SECOND INJECTION
THIRD INJECTION
EXPOSURE

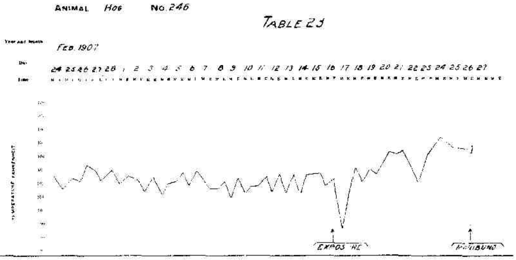

ANIMAL HOG No. 241
TABLE 18
Year and Month.
FEB 1907
Day.
24 25 26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time.
TEMPERATURE FAHRENHEIT.
FIRST INJECTION
SECOND INJECTION
EXPOSURE
FOUND DEAD

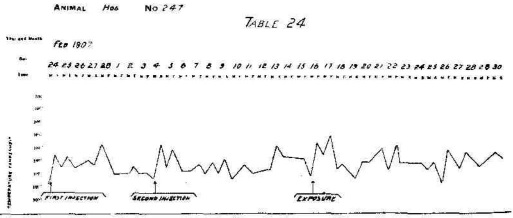

ANIMAL HOG No. 242
TABLE 19
Year and Month.
FEB. 1907
Day.
24 25 26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13
Time.
M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E
TEMPERATURE FAHRENHEIT.
FIRST INJECTION
SECOND INJECTION
FOUND DEAD

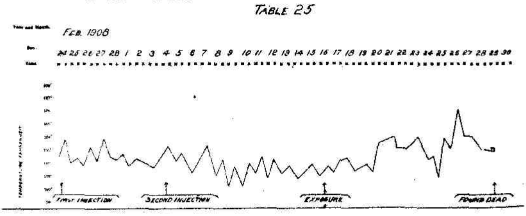

ANIMAL Hog No. 243 (Control)
Table 20
Feb. 1907
Exposed


ANIMAL Hog No. 244
Table 21
Feb. 1907
First Injection
Second Injection
Exposure


ANIMAL Hog No. 245
Table 22
Feb. 1907
First Injection
Second Injection
Exposure


ANIMAL HOG NO. 246
TABLE 23
FEB. 1907
EXPOSURE
MORIBUND


ANIMAL HOG NO 247
TABLE 24
FEB 1907
FIRST INJECTION
SECOND INJECTION
EXPOSURE


ANIMAL HOG NO. 248
TABLE 25
FEB. 1908
FIRST INJECTION
SECOND INJECTION
EXPOSURE
FOUND DEAD

Experience tends to show that 24-hour horse-serum vaccine is not constant as to its protective power when injected subcutaneously in small quantities. The following experiment, indicated in table 26, shows the results obtained from the subcutaneous vaccination of a series of seven healthy hogs with small doses of 24-hour horse-serum vaccine:

TABLE 26.

EXPERIMENTAL VACCINATION.

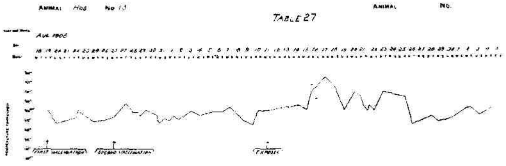
Hog No.	No. of injections of vaccine.	Date and material used, first injection.	Date and material used, second injection.	Time between last injection and exposure.	Result of exposure.
13	2	Aug. 19, 1908. 24-hr. horse 3. 3 cc. Sc.	Aug. 26, 1908. 24-hr. horse 3. 5 cc. Sc.	16 days	Did not take the disease.
15	1	Aug. 21, 1908. 24-hr. horse 4. 4 cc. Sc.		20 days	Moribund September 27. Chloroformed, bled, and posted.
16	1	Aug. 29, 1908. 24-hr. horse 3. 3 cc. Sc.		12 days	Found dead from cholera, September 28, 1908.
17	1	Aug. 24, 1908. 24-hr. horse 3. 3 cc. Sc.		12 days	Moribund September 27. Chloroformed, bled, and posted.
18	1	Aug. 29, 1908. 24-hr. horse 3. 3 cc. Sc.		12 days	Morbid September 27. Chloroformed, bled, and posted.
19	1	Sept. 2, 1908. 30-hr. horse 4. 4 cc. Sc.		8 days	Did not take the disease.
20	Control.				Found dead from cholera, September 26, 1908.
21	1	Sept. 2, 1908. 30-hr. horse 3. 3 cc. Sc.		8 days	Did not take the disease.
24	Control.				Found dead from cholera, October 5, 1908.

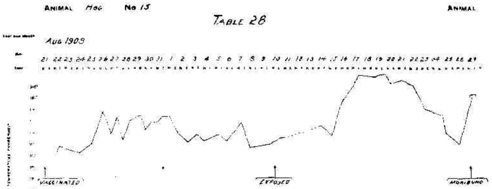
With the exception of hog No. 13, which was exposed September 11, 1908, all these animals were exposed September 10, 1908, by being injected subcutaneously with 3 cc. each of mixed hog-cholera serum from hogs Nos. 6, 9, 10 and 11.

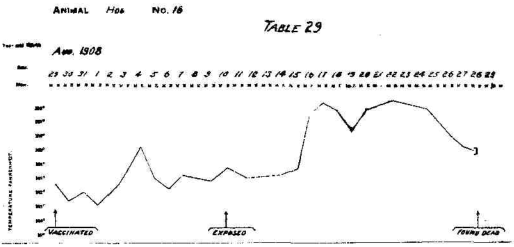
In the above experiment 42⁶⁄₇ per cent. of the vaccinated hogs were saved. All of the vaccinated hogs manifested a strong thermal reaction five to seven days after the exposure, which consisted of the subcutaneous injection of virulent hog-cholera serum. Hogs Nos. 13, 19 and 21, however, all of which showed a temperature reaction of from 106 degrees to 108 degrees, remained bright and active and soon regained their normal condition. In plate 9 is shown a photograph of these three hogs,

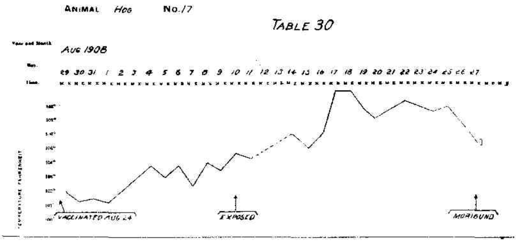
together with the dead control, hog No. 24, which died on October 5. Hogs Nos. 13, 19 and 21 are at present in good, healthy condition. Both control animals, hogs Nos. 20 and 24, upon *post-mortem* showed typical lesions of acute hog cholera. See half-tone showing autopsy of hog No. 24, plate 10.

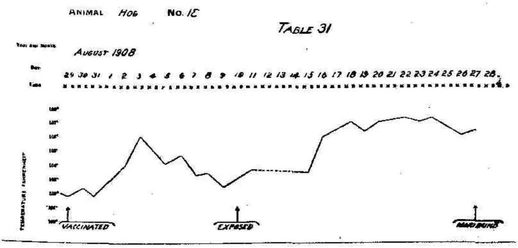
In connection with the above experiment, attention should be called to the following facts: (1) A relatively small quantity of horse-serum vaccine was used in each vaccination; (2) the experimental animals were young pigs, weighing approximately thirty pounds each, which are much more difficult to immunize than are larger hogs; (3) the exposure was rigid, each animal receiving a hypodermic injection of highly virulent serum.

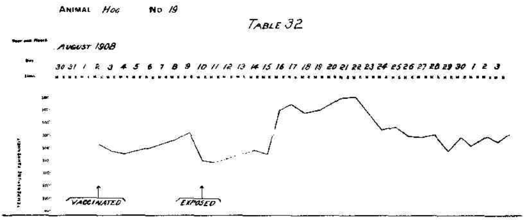


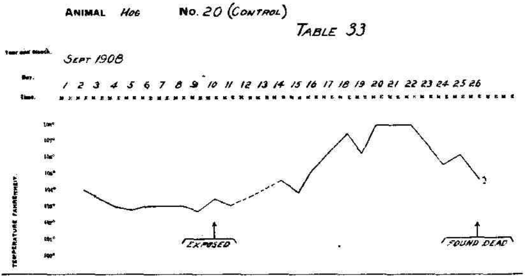


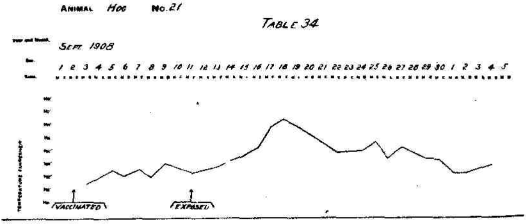

ANIMAL Hog No. 16
TABLE 29
Aug. 1908
VACCINATED
EXPOSED
FOUND DEAD
TEMPERATURE FAHRENHEIT


ANIMAL Hog No. 17
TABLE 30
Aug 1908
VACCINATED AUG 24
EXPOSED
MORIBUND
TEMPERATURE FAHRENHEIT


ANIMAL Hog No. 18
TABLE 31
August 1908
VACCINATED
EXPOSED
MORIBUND
TEMPERATURE FAHRENHEIT


ANIMAL Hog No 19
TABLE 32
AUGUST 1908
30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2 3
VACCINATED
EXPOSED


ANIMAL Hog No. 20 (CONTROL)
TABLE 33
Year and Month.
SEPT 1908
Day.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Time.
TEMPERATURE FAHRENHEIT.
EXPOSED
FOUND DEAD


ANIMAL Hog No. 21
TABLE 34
SEPT 1908
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5
VACCINATED
EXPOSED

A glance at the above temperature charts of the hogs described in the above experiment will show that an apparent temperature reaction followed the vaccination in most cases. It will be noticed that hog No. 21 manifested no evident rise in temperature. Hogs Nos. 13, 15, 16, 17, 18 and 19 all showed distinct temperature reactions five to seven days after vaccination.

A general survey of the whole work with 24-hour horse-serum vaccine indicates that it is not entirely constant and uniform in its protective action. The following preliminary experiment (referred to under "Intravenous *versus* Subcutaneous Inoculation with Horse-serum Virus") suggests the possibility that when the hog-cholera virus is subjected to a shorter residence in the horse than twenty-four hours it may show constant protective properties:

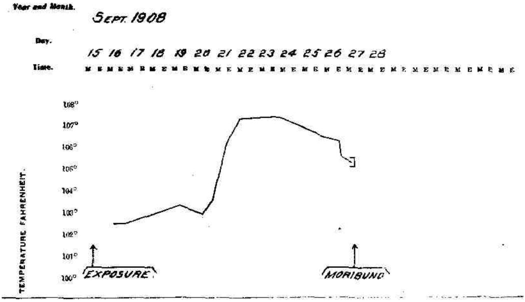
On September 2, 1908, pig No. 22, weight about 30 pounds, was injected subcutaneously with 4 cc. of horse No. 4, 4-hour serum. On September 4, 1908, pig No. 23, in the same litter, with pig No. 22, received subcutaneously 4 cc. of horse No. 3, 4-hour serum. Neither of these animals showed any apparent disturbances after inoculation. On September 15, hogs Nos. 22 and 23, together with control hog No. 26, were injected intravenously, each with 5 cc. of 4-hour horse serum. Hog No. 26, the control, received horse No. 3, 4-hour serum. Neither of the pigs which received the previous subcutaneous injection manifested any deviations from normal conditions as a result of the intravenous injection. Hog No. 26, the control, however, after five days contracted a severe case of the disease and became moribund on September 27, twelve days after

...re chart, table 36.) Lesions in the heart ...found in the *post-mortem* of this animal appear in

...s. 22 and 23 on September 26 were placed in an infected pen ... sick hogs, Nos. 26 and 8. They were fed fresh viscera of hogs Nos. 17 and 18, which had died from cholera. Both animals showed a rise in temperature of from two to three degrees five days after eating the infected viscera. (See temperature charts, tables 34 and 35.) No other indications of a disturbance followed. These two animals are at present under constant exposure in an infected pen and appear heathy in every way. The photograph below (plate 13) shows hogs Nos. 22 and 23, on October 5, together with dead control hog No. 26, and chronic case, hog No. 8, with which they had been confined.

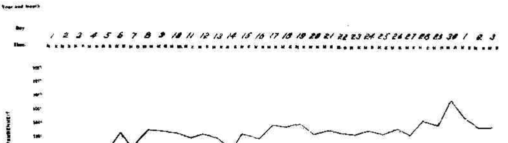
ANIMAL Hog No. 26

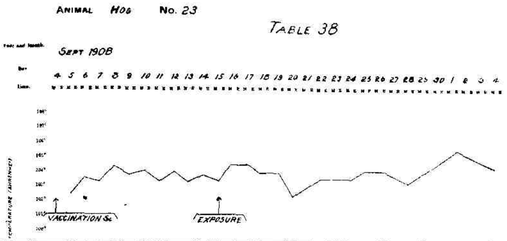
TABLE 36



ANIMAL Hog No. 22

TABLE 37





It appears logical to continue the experiments with horse-serum hog-cholera vaccine for the purpose of determining whether some period of residence in the horse less than twenty-four hours will not sufficiently attenuate the hog-cholera virus so that it will exhibit protective properties with more reasonable uniformity than does the 24-hour serum vaccine. The results of these preliminary experiments suggest that under some conditions 4-hour horse serum is pathogenic to the hog, while a residence of twenty-four hours in the horse tends to modify these pathogenic properties of the virus to such an extent that it does not produce the disease in a healthy hog. The foregoing experiments show that 2-hour horse serum produces the disease when injected in small quantities subcutaneously; and that 4-hour horse serum does not cause infection when injected in small quantities subcutaneously, but does produce acute hog cholera when injected intravenously in reasonable doses. It has also been shown that 5-hour and 7-hour horse serum does not produce infection when injected subcutaneously. Moreover, the subcutaneous inoculation of 4-hour horse serum uniformly produces immunity, according to preliminary experimentation. The 24-hour horse serum has been shown quite definitely to be non-virulent in character and attenuated to such a degree that its vaccinating properties are not constant. If such conditions are true the practical question to be solved is, After what period of residence in the circulatory system of the horse does the hog-cholera virus become most efficient as a vaccine without producing infection? The problem resolves itself into the correct standardization of the horse-serum vaccine.

In connection with this work a limited amount of field-work is at present in progress in various parts of the state of Kansas. Extensive experimentation must be conducted and considerable time must necessarily be employed to definitely prove the reliability of the method of vaccination.

VII.

DURATION OF IMMUNITY.

Experimental evidences warrant the statement that a hog once immunized to the disease of hog cholera will retain the protection for a period of several months. Thus the successfully vaccinated or immunized hog may be kept with no risk of infection for a period long enough to fatten and prepare the animal for market. The following data relative to this point are given as experimental evidence:

Hog No. 1, grade English Yorkshire, female, weight about 60 pounds, received the following treatments: January 12, 1908, injected Sc. 8 cc. bay mare 48-hr. serum, which was drawn June 29, 1907; January 31, 1907, injected Sc. 5 cc. crippled mare 24-hr. serum, drawn March 25, 1907; February 17, 1908, injected Sc. 5 cc. gray horse 24-hr. serum, drawn April 4, 1907; February 29, 1908, injected Sc. 2 cc. hog-cholera serum, hog No. 259, drawn May 16, 1907; March 30, 1908, injected Sc. 6 cc. hog-cholera serum, hog No. 264, drawn May 30, 1907; April 10, 1908, fed viscera and blood from dead hogs, Riley strain; July 25, 1908, placed in infected pen with hogs Nos. 9 and 10; August 19, 1908, injected Sc. with 5 cc. horse No. 3 4-hour serum; September 28, 1908, fed viscera and placed with hogs sick and dying from hog cholera.

Hog No. 2, grade Duroc-Jersey, boar, weight about 80 pounds, received the following treatments: January 12, 1908, injected Sc. with 8 cc. bay mare 6-hour serum, drawn April 14, 1907; May 13, 1907, hog No. 264 received Sc. 10 cc. of bay mare 6-hour serum, and under conditions of isolation eight days after developed acute hog cholera. Hog No. 264 died on the seventeenth day after inoculation. Hog No. 2 showed rise of temperature (see table 39) and loss of appetite five days after inoculation, but after eleven days again appeared normal. On January 31 and February 17, hog No. 2 received injections Sc. with the same material with no apparent effects. February 29, hog No. 2 received Sc. 0.2 gm. dried hog-cholera serum from hog No. 264, drawn May 30, 1907; March 31, hog No. 2 received Sc. 6 cc. hog-cholera liquid serum from hog No. 264. This animal received the same exposures as hog No. 1, described above.

Hogs Nos. 3 and 4, grade Yorkshire, weight about 40 pounds each: On February 29, hog No. 3 received Sc. 0.2 gm. dried hog-cholera serum from hog No. 259, drawn May 16, 1907. Evidences of cholera followed the inoculation. On the same date hog No. 4 received Sc. 2 cc. of hog-

cholera serum from hog No. 264, drawn May 30, 1907. After six days evident thermal disturbances followed. The temperature again became normal twelve days after inoculation. Hogs Nos. 3 and 4 each received the same exposures, as follows: March 30, 1908, injected Sc. with 6 cc. of hog-cholera serum from hog No. 264, drawn May 30, 1907; April 10, fed viscera from hogs dead from cholera; May 1, fed hog-cholera blood and viscera from Riley outbreak; July 4, 1908, fed viscera and blood of hog-cholera hogs, Riley outbreak; July 25, placed in infected pen with sick hogs Nos. 9 and 10; August 18, injected Sc. with 5 cc. horse No. 3, 4-hour serum; September 28, fed hog-cholera viscera and placed in infected pen with hogs sick and dying from hog cholera.

Hog No. 7, white sow, weight on May 25 about 60 pounds: July 1, 1908, injected Iv. with 6 cc. hog-cholera serum, Riley outbreak. No immediate results appeared from inoculation. After several days, the animal gave evidence of a light chronic case of cholera, coughed some and showed some loss of flesh. July 6 this hog was again in normal condition. July 4, fed hog-cholera viscera and blood, Riley outbreak; July 25, placed in infected pen with sick hogs Nos. 9 and 10; August 18, injected Sc. with 5 cc. horse No. 3 4-hour serum; September 28, fed hog-cholera viscera, Norwood outbreak, and placed in infected pens with hogs sick and dying from the disease.

All of the hogs described above were immunized against the disease and are in excellent condition at the present time. (See half-tone, plate 14.)

The methods used in immunizing these hogs are unimportant in this connection. These animals, however, were immunized by the injection of hog-cholera virus, once highly virulent, but attenuated by age at the time of inoculation, and by exposure to the Riley strain of hog cholera, which was very chronic in character. The probable approximate date of acquiring immunity in each of these animals may be placed as follows:

Hog No. 1, during February, 1908.
Hog No. 2, during January, 1908.
Hog No. 3, during March, 1908.
Hog No. 4, during March, 1908.
Hog No. 7, during June, 1908.

These hogs have for the last three months been under conditions of intermittent exposure to hog cholera and are at present confined in pens infected with the acute type of the disease. In each case the duration of immunity has extended over the following periods:

Hog No. 1, approximate duration of immunity, seven months.
Hog No. 2, approximate duration of immunity, eight months.

Hog No. 3, approximate duration of immunity, six months.
Hog No. 4, approximate duration of immunity, six months.
Hog No. 7, approximate duration of immunity, three months.

VIII.

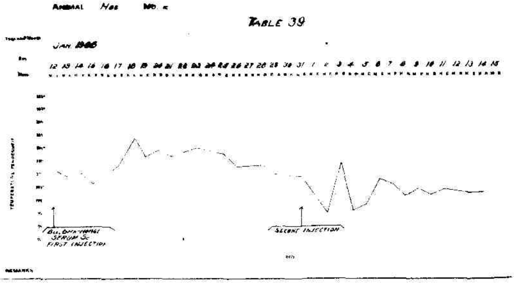
THE KEEPING QUALITIES OF HOG-CHOLERA VIRUS.

A limited amount of data has been obtained relative to the duration of virulence of hog-cholera serum. The observations which were made are as follows:

(a) Liquid Hog-cholera Serum.

On January 12, 1908, hog No. 2 was injected Sc. with 8 cc. of bay mare 6-hour serum. This 6-hour serum was drawn in April, 1907; on May 13, 1907, 10 cc. of this was injected subcutaneously into hog No. 264. Hog No. 264, after an incubation period of eight days, developed acute hog cholera and was moribund seventeen days after inoculation. Hog No. 2, which received 8 cc. of this virus, showed some disturbance in temperature and appetite five days after inoculation. These symptoms soon disappeared, however, showing the attenuated condition of the material. (See table 39.) Subsequent injections and exposures suggested that the attenuated virus served to immunize the animal against disease.

Hog No. 4, small white pig, weight about 40 pounds, on Februbary 29 received Sc. 2 cc. of virus from hog No. 259. Hog No. 259 had a severe case of the disease and was bled on May 16, 1907. Some reaction followed the injection of hog No. 3, but infection did not follow.



(b) Dried Hog-cholera Serum.

Hog No. 2, on February 29, received 0.2 gm. of dried hog-cholera serum from hog No. 259, drawn May 16, 1907. This dried material was found to be virulent by the inoculation of a healthy hog. This was dried

by exposure to a temperature of 37° C. No results followed this injection. The experiment is not conclusive, because of the fact that hog No. 2 had received previous inoculations of bay mare 6-hour serum, which had probably conferred immunity.

Hog No. 3, a small white pig, weighing about 35 pounds, on February 29 received 0.2 gm. of dried hog-cholera virus from hog No. 259, drawn May 16, 1907. No apparent reaction or evidences of infection followed this injection.

From the above experiments we find the following results:

(*a*) *Liquid Serum.*—Hog No. 2 was not infected by inoculation on July 12, 1908, with material which was obtained in April, 1907, and which produced the disease in a healthy pig in May, 1907; the virus had therefore lost its virulency after a period of nine months.

Hog No. 4 showed only a slight reaction when inoculated on February 29, 1908, with hog-cholera serum obtained from a severe case on May 16, 1907. Again after a period of nine months this virus had become attenuated.

(*b*) *Dried Serum.*—Hog No. 3 gave no evidence of disease after injection on February 29, 1908, with hog-cholera serum which was obtained on May 16, 1907, in a highly virulent condition, and was dried at a temperature of 37° C.

From the above limited amount of data (experiment with hog No. 3) the suggestion follows that neither hog-cholera virus nor a serum vaccine could be protected from deterioration by preserving it in a dry condition.

IX.

KEEPING QUALITIES OF HORSE-SERUM VACCINE.

The assumption could reasonably be made that horse-serum vaccine would probably retain its protective properties as long as does the virulent hog-cholera serum. The following recorded results, in so far as they extend, give some information on this point:

TABLE 37.

KEEPING QUALITIES OF HORSE-SERUM VACCINE.

Hog No.	Date of vaccination.	Character of vaccine used.	Result of vaccination.	Date vaccine was obtained.	Age of vaccine.
19	Sep. 2, 1908	30-hr. horse serum.	Successful.	Aug. 20, 1908	One week and six days.
21	Sep. 2, 1908	24-hr. horse serum.	Successful.	Aug. 24, 1908	One week and two days.
22	Sep. 2, 1908	24-hr. horse serum.	Successful.	Aug. 19, 1908	Two weeks.
13	Aug. 19, 1908	24-hr. horse serum.	Successful.	Jul. 18, 1908	Four weeks and four days.
23	Sep. 2, 1908	4-hr. horse serum.	Successful.	Jul. 17, 1908	Six weeks and four days.

The horse serum vaccines used in the inoculations indicated in table 37 were kept in a refrigerator, temperature approximately 12° C.

SUMMARY.

From the results which have thus far been obtained in the present work and from those of other investigators which have been reviewed in the preceding pages, the following conclusions seem warranted:

1. *Bacillus cholera suis* is not the etiological factor in all forms of hog cholera.

2. *Bacillus cholera suis* is possibly a variety of the common intestinal parasitic organism, *Bacillus coli communis.*

3. The specific cause of the "filterable virus form" of hog cholera appears to be some living organism, possibly ultramicroscopic, possibly capable of passing through a fine porcelain filter in some disintegrated state.

4. The filterable virus of hog cholera cannot be artificially cultivated in normal hog-serum broth in the abdominal cavity

of the rabbit in the collodium sac, according to the method used by Nocard and Roux in artificially cultivating the organism which they associate with contagious pleuropneumonia in cattle.

5. The simultaneous method of vaccination is efficient but is not practical because of its expense and the possible danger attending its use.

6. The ordinary laboratory and domesticated animals are *not susceptible to the filterable virus of hog cholera.*

7. Virulent hog-cholera serum exerts a toxic influence upon a healthy horse when injected intravenously.

8. Normal hog-cholera serum or virulent hog cholera serum does not appear to produce an anaphylactic reaction when injected intravenously into the horse.

9. The 2-hour horse serum (drawn from the horse two hours after the animal has received, intravenously, approximately 150 cc. of hog-cholera virus) when injected subcutaneously into the healthy hog in small quantities produces an acute form of the disease.

10. The 4-hour horse serum (blood serum from a horse drawn four hours after the animal has received intravenously approximately 150 cc. of virulent hog-cholera serum) when injected into healthy hogs intravenously produces acute hog cholera.

11. *The 4-hour horse serum, under certain conditions at* least, when injected subcutaneously in small doses into healthy swine does not produce infection.

12. The 5- to 7-hour horse serum when injected subcutaneously into the healthy hog does not produce the disease.

13. The 24-hour horse serum (drawn twenty-four hours after the animal has received intravenously approximately 150 cc. of hog-cholera serum), in comparison with 4-hour horse serum, *shows attenuated properties.*

14. The 24-hour horse-serum vaccine injected subcutaneously and intravenously, and 4-hour horse-serum vaccine when injected in small quantities subcutaneously, act as preventives against hog cholera. The 24-hour horse serum, however, is not constant in respect to its protective properties.

15. Acquired immunity against hog cholera extends over a period of from three to eight months.

16. A rough estimate shows that horse-serum vaccine can be prepared at a relatively low cost.

17. Virulent hog-cholera serum in the liquid form becomes attenuated after a period of nine months when kept at a temperature of approximately 10° C. to 15° C.

18. Virulent hog-cholera serum dried under aseptic conditions at a temperature of 37° C. becomes attenuated after a period of eight months.

19. Horse-serum vaccine retains its protective properties for at least a period of six weeks when kept at an approximate temperature of 10° C. to 15° C.

PROPOSED CONTINUATION OF PRESENT INVESTIGATION.

At the present time, active field-work with horse-serum vaccine in various parts of the state of Kansas is being undertaken. This work will be continued until the winter months, when the reports from the various field experiments will be secured and arranged in definite form.

At the Station, various experiments will be conducted with horse-serum vaccines drawn at different periods of time after the intravenous injection of the horse with the hog-cholera virus. As many series of hogs as opportunity will permit will be used under various conditions in testing the above vaccines.

Comparative leucocyte counts will be made of the blood of the horse before and after the intravenous injection of normal and hog-cholera serum.

Data will be obtained relative to the influence of one or more treatments of the horse with hog-cholera serum. This must be determined in order to be able to standardize correctly the horse-serum vaccine.

Continued observations will also be made upon duration of acquired immunity, possible immunity by inheritance, and the keeping qualities of hog-cholera virus and horse-serum vaccine.

Within the next few months it is expected that this bulletin will be followed by one which will review the present work in a more popular manner. The writer is aware of the fact that the contents of this bulletin consist of facts which are perhaps too technical to be of more than ordinary interest to the farmer,

but it must be understood that in any investigation a certain amount of experimental and technical work must be accomplished before the final successful, practical product is obtained.

In the next bulletin the results of the field-work which is being conducted with the horse-serum vaccine will also be given.

PLATE 1. Hog yards for experimental hog-cholera work.
Kansas Experiment Station

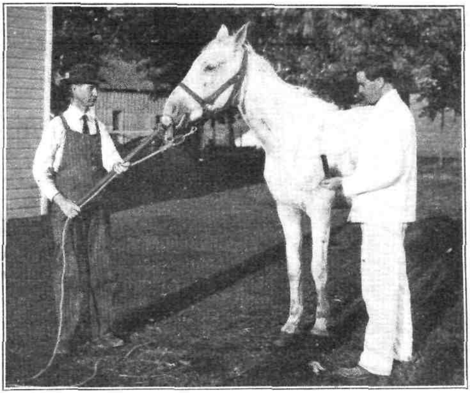


PLATE 2. Intravenous injection of horse with hog-cholera virus.

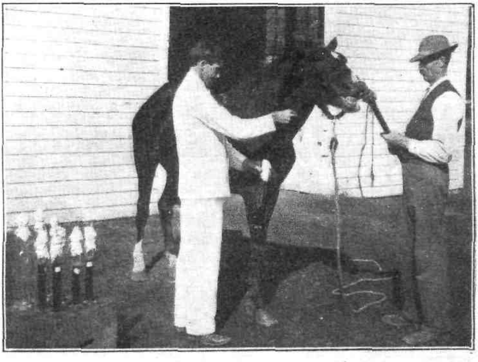


PLATE 3. Bleeding the horse.

PLATE 4. Spleen from original hog, Norwood, Kan., about twice the size of normal spleen of seventy-five-pound pig.

PLATE 5. Ulcers in cæcum of original hog, Norwood, Kan.

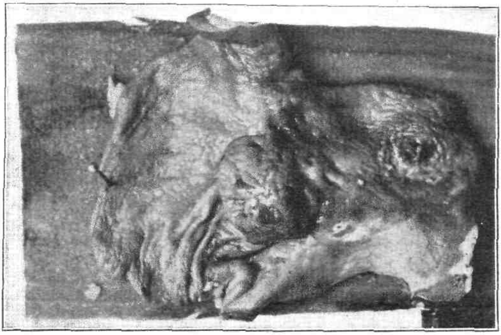


PLATE 6. Ulcers in cæcum of hog No. 9.

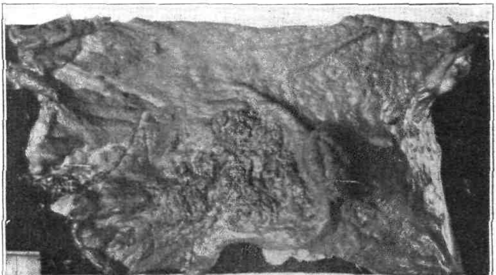


PLATE 7. Ulcers in cæcum of hog No. 10.

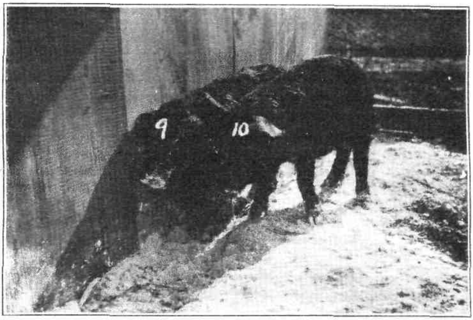


PLATE 8. Hogs Nos. 9 and 10, August 1, 1908. Fifteen days after inoculation.

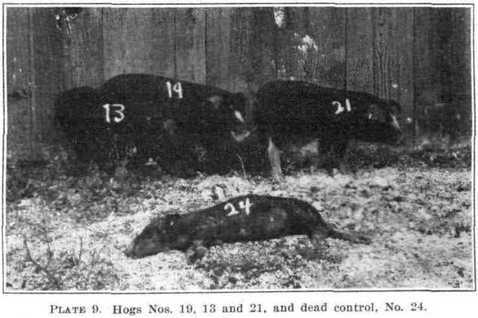


PLATE 9. Hogs Nos. 19, 13 and 21, and dead control, No. 24.

PLATE 10. Autopsy, hog No. 24, showing enlarged and hemorrhagic mesenteric glands, ulcerated cæcum, enlarged spleen, hemorrhagic areas on peritoneum, subcutaneous and muscular tissues, blood extravasations in heart muscle and hepatized and hemorrhagic portions of lungs.

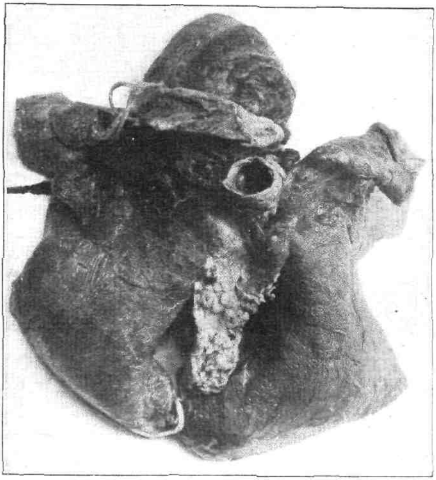


PLATE 11. Heart and lungs, control hog No. 26, hepatized and hemorrhagic areas in both lungs, heart covered with blood extravasations and a fibrinous exudate.

PLATE 12. Kidney, control hog No. 26, showing petechiæ.

PLATE 13. Hogs Nos. 22 and 23, with chronic case No. 8 and dead hog No. 24.

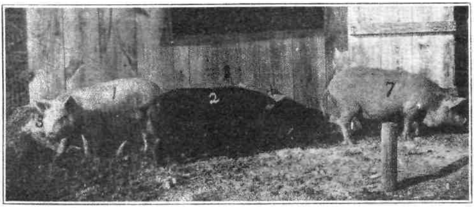


PLATE 14. Immune hogs Nos. 1, 2, 3, 4 and 7—immunity having extended over a period of from three to eight months.